

SHORT COMMUNICATION

The effects of temperature on egg development and web site selection in *Nephila clavipes*

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Abstract. Temperature affects metabolic rate and egg development in mature female spiders. For temperate species, declining temperatures toward the end of the reproductive season may limit reproductive output, particularly for late-maturing females. Although spiders are known to alter their web-site preferences in response to temperature, it is unknown whether females can use web placement to overcome low temperatures that prohibit reproduction and thus extend their reproductive time frame. I surveyed web temperatures for female *Nephila clavipes* (Linnaeus 1767) to compare female web sites and control sites at the beginning and end of the reproductive season in order to assess whether females change their web preferences in response to declining temperatures. Survey data showed that the web sites chosen by females at the end of the reproductive season have a higher minimum temperature than sites occupied during the early season. In a laboratory experiment, I addressed whether a low but biologically relevant temperature affects egg development and the female's ability to oviposit in *N. clavipes*. Females kept at 16° C failed to oviposit and showed signs of slowed egg development. Thus this preliminary study suggests that females may be able to protect themselves against temperatures that are prohibitively low for reproduction, but further experiments should explore the effects of temperature on egg development and web-site selection in this species.

Keywords: Behavioral thermoregulation, metabolism, seasonal constraints, size

A spider web is a microenvironment with a unique set of abiotic and biotic conditions that affect the survival and reproductive success of the web-owner (Agnarsson 2003; Rittschof & Ruggles 2010). Because web site choice is so critical to individual fitness, many researchers have examined the criteria that female spiders evaluate when choosing a web site (e.g., Elgar et al. 1996; Heiling 1999; Adams 2000; Bilde et al. 2002). Web temperature is one abiotic factor that is known to affect prey availability (Herberstein & Fleisch 2003), the web owner's metabolic rate and activity level (Lubin & Henschel 1990; Li & Jackson 1996), as well as web site selection in certain species (Henschel et al. 1992; Voss et al. 2007). In an environment where temperature is highly variable over time and space, adult females, whose egg development is temperature-dependent, will benefit if they can moderate their body temperature through web site selection. Although past studies show that female spiders have thermal preferences, few studies have attempted to address whether females can successfully overcome suboptimal temperatures through web site selection, and thus extend the time period during which they are reproductively active.

In the temperate (e.g., Florida) populations of the golden orb-web spider *Nephila clavipes* (Linnaeus 1767) (Araneae, Nephilidae), low temperatures at the end of the season can result in female death prior to successful oviposition (Higgins 2000). Because individuals have only a single season to reproduce, the possibility that females can prolong their lifespan or reproductive period through web site placement has important fitness implications. I surveyed mean and minimum web temperature for females in the field at the beginning and end of the season to assess how female web

placement affects body temperature and whether site preferences change toward the end of the season as temperatures decline. In addition, I performed a laboratory experiment to test the effects of late-season minimum temperatures on egg development in adult *N. clavipes*. Together these data lend support to the hypothesis that seasonal decline in temperature limits egg development and affects web site selection in *N. clavipes*. By changing web site preferences, females may be able to overcome late season reproductive constraints behaviorally.

I measured temperatures at female web sites early (July) and late (October) in the 2010 reproductive season. At both times of the season, I sampled the first 20 mature female webs found within 10 m of a 200 m linear transect in the Ordway-Swisher Biological Station. For each web site, I established a control site at a height of 80 cm and a distance of 10 m from the web site in a cardinal direction chosen at random. At both web and control sites, I sampled temperature every 5 min for 72 h using data loggers (described below) to determine mean and minimum temperatures. The 72 h time period is within the range of a typical web tenure for an adult *N. clavipes* in this population (Rittschof & Ruggles 2010). The 20 early season web and control sites were sampled in three blocks: 16–19 Jul 2010 ($n = 7$ webs and 7 controls), 21–24 Jul 2010 ($n = 7$ webs and 7 controls), and 27–30 Jul 2010 ($n = 6$ webs and 6 controls). Similarly, I sampled webs in the late season in two blocks: 5–8 Oct 2010 ($n = 10$ webs, 10 controls) and 10–13 Oct 2010 ($n = 10$ webs, 10 controls). Finally, in order to determine whether some web sites chosen in the early season become unsuitable in the late season due to temperature changes, I re-sampled the 20 early-season web sites (but not the controls) during the late season.

In order to measure web site temperature (a proxy for female body temperature) at a fine spatial scale over an

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extended time period, I developed a physical model (Bakken 1992) for a female *N. clavipes* abdomen and embedded an iButton® temperature data logger (Maxim Integrated Products, Sunnyvale, CA) within the model. The model was a piece of brown clay (Van Aken International, Rancho Cucamonga, CA). After I embedded the data logger, the disc-shaped model was approximately 21 mm in diameter and 8 mm deep with the data logger embedded 3 mm in clay. I verified that the brown clay mimics temperature fluctuations in a female abdomen by comparing thermocouple measurements from the abdomen of a dead *N. clavipes* to a similar-sized piece of brown modeling clay over a 24 h period (5 min intervals). The temperature distributions were not significantly different across the sampling period (Kolmogorov-Smirnov Test, $D = 0.027$, $P = 0.99$).

When sampling web and control sites, I mounted each model onto a pole (1 cm in diameter), positioning the model within 5 cm of the dorsal side of the female spider at each web site. Using this method I could approximate the temperature changes experienced at the web hub, which is the resting position for female *N. clavipes* during the day and night. This approach, however, did not allow me to capture the effects of web or body orientation on body temperature, which are other means of behavioral thermoregulation in spiders (Herberstein & Heiling 2001; Ramirez et al. 2003). At control sites, I mounted data loggers on poles 80 cm from the ground.

First I tested whether web site selection is affected by thermal characteristics by comparing the mean and minimum temperatures at web and control sites using a nested ANOVA with sampling date nested within season. For this analysis, I omitted the late-season data from re-sampled early-season webs. There were significant effects of season and sampling date on mean web temperature. However, there was no evidence of temperature-based web site selection: there was no difference between web sites and control sites in either mean temperature ($n = 20$ webs and 20 control sites at two times of the season, Season: $F_1 = 1416.7$, $P < 0.0001$; Sampling date: $F_3 = 156.8$, $P < 0.0001$; web versus control site: $F_5 = 1.2$, $P = 0.48$) or minimum temperature ($n = 20$ webs and 20 control sites at two times of the season, Season: $F_1 = 11830.9$, $P < 0.0001$; Sampling date: $F_3 = 926.5$, $P < 0.0001$; web versus control site: $F_5 = 1.2$, $P = 0.33$). Thus although web temperatures change on a daily basis and decrease as the season progresses, on a given day, female web temperatures do not differ from sites within the same area chosen at random. One explanation for the lack of significant temperature difference between web and control sites within a single season period could be that the 10-m difference in location between web and control sites (see above) failed to capture the range of temperature variation found along the sampling transect.

In order to test whether spiders select webs at the beginning and end of the season on the basis of temperature profile differences, I re-sampled the 20 early-season web sites simultaneously with the 20 web sites in the late season. There were no spiders occupying the early season web sites at the time that they were re-sampled in the late season. For this analysis I constructed an ANOVA with season (i.e., whether sites were occupied in the early or late season) nested within sample date. In this analysis, there was no significant effect of

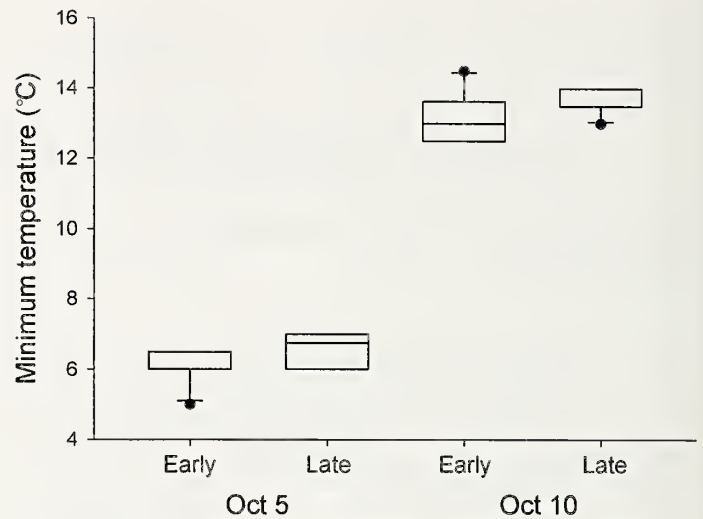


Figure 1.—Minimum web temperatures measured on either 5 Oct 2010 or 10 Oct 2010, comparing webs preferred in the early season (early, $n = 20$ total) to webs preferred in the late season (late, $n = 20$ total). The lower and upper sides of the boxes represent the 25th and 75th percentiles respectively, and horizontal lines through the middle of the boxes are the medians. Vertical lines extending from the bottom and top of the boxes show the 10th and 90th percentiles respectively, and the data points shown are values beyond the 10th and 90th percentiles.

season on mean temperature, but there was a significant effect of sample date (season: $F_2 = 2.7$, $P = 0.083$; sample date: $F_1 = 1064.8$, $P < 0.0001$). In addition, season had a significant effect on minimum temperature. Web sites occupied by females in the early season had lower minimum temperatures in the late season than sites occupied by females in the late season (mean_{early} = 9.6° C, SE = 0.8° C; mean_{late} = 10.1° C, SE = 0.8° C; sampling date: $F_1 = 2074$, $P < 0.0001$, season occupied: $F_2 = 1.19$, $P = 0.0104$, Fig. 1). These results suggest that females may respond to temperature when selecting web sites.

In addition to assessing field web site temperatures, in a laboratory experiment I examined the effects of temperature on egg development. I used changes in abdomen size to estimate egg development in females. To verify that variation in ovary size (a measure of the degree of egg development; Trabalon et al. 1992) is correlated with female abdomen size, I analyzed the relationship between abdomen size and ovary mass in wild-caught females. I collected 16 females in Alachua County, Florida between 27 July 2010 and 12 September 2010 and dissected them within 1 day of collection. I placed females in a -20° C freezer for 7 min and photographed and measured female abdomen height (the dorsal-ventral height of the abdomen measured just posterior to the epigynal slit: Vincent & Lailvaux 2006; Rittschof & Ruggles 2010), following Rittschof (2011). I dissected the ovaries out of the abdomen in 10 mM phosphate-buffered saline and preserved them in 95% ethanol. Prior to weighing, I dried ovaries in an oven at 37° C for 30–60 min, depending on size. There was a strong positive relationship ($R^2 = 0.80$, $P < 0.0001$) between log-transformed ovarian mass (prior to transformation: range: 0.0006–0.2987 g, mean = 0.095 g, SE = 0.03 g) and abdomen height (range: 4.4–11.9 mm, mean = 9.2 mm, SE = 0.55 mm),

supporting my use of adult female abdomen height as a measure of egg development in *N. clavipes*.

Once I had established that abdomen height is an adequate measure of egg development, in a laboratory setting I tested how long-term exposure to low temperature affects changes in female abdomen size, egg development, and the ability to oviposit. I predicted that when kept at low temperatures, female abdomen height would either fail to increase over time, or if it did increase, that this change would not correspond to an increase in ovary size. Finally, I predicted that females kept at low temperatures would fail to reach oviposition. In order to control for laboratory artifacts, I monitored a control group of spiders kept at a warmer temperature (24° C). I collected 16 mature females from the Ordway-Swisher Biological Station in Melrose, Florida (Putnam County, latitude 29°42'32.4"N, longitude 82°2'60.0"W) on 25 Aug 2009. I assigned females to either the cold or warm temperature treatment at random. Because females are typically mated immediately after their maturation molt (Christenson et al. 1985), I assumed mature females were non-virgin. If some females were unmated at the time of collection (which would impact egg development rate: Trabalon et al. 1992), this should not have changed the major results of the experiment because females were assigned to the temperature-controlled rooms ($n = 8$ females per room) at random. In the cold treatment I kept females at 16° C, which is approximately the minimum daily mean temperature during the month of October (Fig. 2), the last month of the season with an appreciable number of adult females still alive and presumably attempting to produce egg clutches. Females kept in the warmer control room were held at 24° C, which is near the mean temperature for the month of September, when females are still observed to lay eggs in the field (Rittschof & Ruggles 2010). Prior to initiating the experiment, there were no significant differences in abdomen height comparing 16° C and 24° C females (t -test, $t_{14b} = 1.44$, $P < 0.25$).

From 27 Aug 2009 to 30 Sep 2009, I housed mature female spiders in individual cages (30.5 cm cubes; Bioquip, Rancho Dominguez, CA) in one of the two temperature-controlled rooms (approximately 3.1 m × 4.6 m × 2.4 m) maintained at a 12 h light/dark cycle. Humidity could not be controlled within the rooms. Cages were small relative to typical *N. clavipes* web sizes (average web width is about 99 cm: Vincent & Lailvaux 2006), but given 24 h within these cages in a field setting, all females built prey-capture webs. Within each room I rotated the females' positions every other day in order to control for variation in conditions within the room. I measured the abdomen height for each female from digital photographs each day following Rittschof (2011), and I fed females 1 approximately 2.5 cm long mealworm each day that they had an intact prey-capture web. Previous studies (Rittschof 2010, 2011) show that caged females kept in outdoor conditions and fed the same diet successfully laid multiple egg clutches at ~25 day intervals. When a female died in the 16° C room, I dissected her abdomen following the protocol for wild females (see above). I did not evaluate female ovary sizes from the 24° C room because, unlike females kept at 16° C, some of these females laid eggs (see below) and thus were at various reproductive stages at the time of death. Overall, 38% of females died within the time frame of the experiment. For

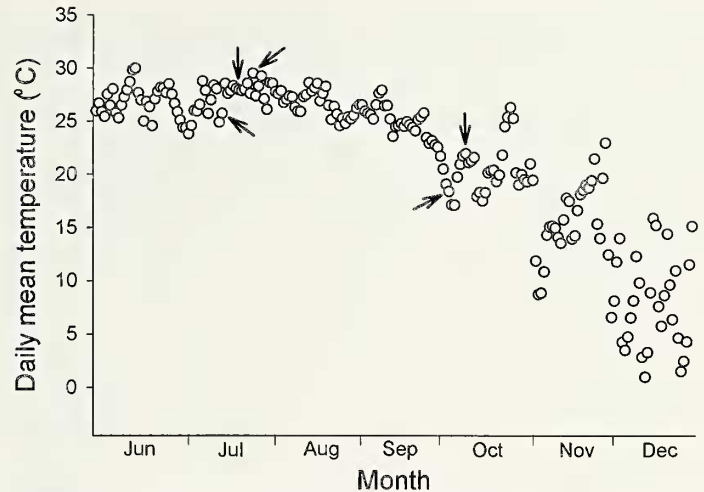


Figure 2.—Daily mean temperatures from the Putnam Hall weather station within the Ordway-Swisher Biological Station. Means were calculated from 96 temperature readings per day taken at 2 m height (the approximate height of webs included in this study). Data were accessed from the Florida Automated Weather Network (University of Florida Institute of Food and Agricultural Sciences Extension). Arrows mark sampling dates (the first of three consecutive days of sampling). 16 Jul 2010, 21 Jul 2010, and 27 Jul 2010 in the early season and 5 Oct 2010 and 10 Oct 2010 in the late season.

those that did not, I kept females in the same treatment conditions until they died in order to calculate a measure of reproductive lifespan, which I defined as the length of time between collection from the wild and death.

There were notable effects of laboratory conditions. Reproductive lifespan across all laboratory animals ($n = 8$ females per room; mean = 27.3 days, SE = 1.8 d) was much shorter than the maximum reproductive lifespan in temperate populations of *N. clavipes* (as long as 4 months: C. Rittschof personal observation; Brown 1985; Christenson et al. 1985). Female performance, indicated by the percentage of days that females successfully built prey-capture webs and attempted to feed, was similar between the two treatment rooms (16° C: $n = 8$, mean = 52.6%, SE = 10.8%; 24° C: $n = 8$, mean = 49.4%, SE = 12.0%; two-tailed t test, $t_{14} = 0.041$, $P = 0.84$), although performance seemed low compared to females kept in outdoor cages or unrestrained females in the wild (C. Rittschof, personal observation).

Despite shorter overall reproductive lifespan in the laboratory, mean reproductive lifespan was significantly longer for females kept at 16° C versus 24° C (mean_{cold} = 31 d, SE = 2.2 days; mean_{warm} = 24 days, SE = 2.2 d; two-tailed t test, $t_{14} = 5.5$, $P < 0.034$). Prolonged reproductive lifespan in the colder room is in agreement with a fundamental prediction of metabolic theory: metabolism decreases with temperature, resulting in increased lifespan (Brown et al. 2004). Thus this result provides evidence that the low temperature treatment caused metabolic changes in the spiders beyond laboratory artifacts.

Results suggest that decreased temperature may affect female egg development and propensity to oviposit. In the 24° C room, three females successfully laid egg clutches over the experimental period, and one female laid two clutches of

eggs within this time span. In the 16° C room, no females successfully laid eggs over the entire experimental time period (the last female that died was monitored for 38 days). The differences in the occurrence of oviposition were not statistically significant ($n = 8$ females per room, Fisher's Exact Test, $\chi^2 = 3.7$, $P = 0.1$). Even though females failed to lay eggs in the 16° C room, female abdomen height increased over the course of the experiment ($n = 8$; nested ANOVA with time nested within female identity, $F_{24,175} = 14.2$, $P < 0.0001$). Maximum female abdomen height in the 16° C room was not significantly different from the 24° C room (16° C room, mean = 9.5 mm, 24° C room, mean = 8.5 mm; $t_{14} = -1.74$, $P < 0.10$), and in contrast to wild-caught females, abdomen height for females kept at 16° C was not positively correlated with ovary mass ($n = 7$ females because one cold-room female had necrotic ovaries; mean_{AB} = 7.7 mm, range: 4.1–11.0 mm; mean_{OV} = 0.07 g, range = 0.002–0.14 g; $F_{1,5} = 1.59$, $P < 0.26$), which suggests that abdomen height is not a valid proxy for ovary development under all conditions. These experimental sample sizes are small, however given the predicted strength of the relationship between abdomen height and ovary mass from field data ($R^2 = 0.8$), and the broad range of abdomen sizes represented in the data set, the regression analysis has a statistical power greater than 0.8 to detect a significant correlation between abdomen size and ovary mass.

Because increased ovary mass is the result of the addition of yolk proteins to eggs (Trabalon et al. 1992), one possible explanation for difference between females housed at 16° C and wild-caught females is that females kept at cold temperatures did not efficiently convert the nutrients they consumed into egg protein as a result of decreased metabolism at low temperature (Li & Jackson 1996; Gillooly et al. 2001; Ladyman et al. 2003). The data suggest that the seasonal decline in temperatures (temperatures drop below 16° C while mature females are still alive, Fig. 2) may have implications for female reproductive success. Egg development could slow or stop due to low temperatures, limiting total reproductive output for late-maturing females (Higgins 2000).

Although the current study suggests that seasonal low temperatures may impede female reproduction in *N. clavipes*, the results are not conclusive. For example, the lack of a correlation between ovary mass and abdomen size in the 16° C laboratory females may be due to laboratory artifacts or small sample sizes. Future studies should assess ovary development at various times throughout the experimental period as opposed to at a single time point at the end. The results presented here suggest that further work is needed to clearly capture the effects of temperature on reproduction in this species.

In summary, the laboratory experiment provides some evidence for temperature-dependent egg development in *N. clavipes*. When females are kept at cold temperatures, abdomen height increases over time, but this increase does not correspond to ovary development, two variables that are highly correlated in wild-caught females. Temperature-dependent egg development may explain the shift in web site preferences at the end of the season when temperature decreases and becomes more variable (Fig. 2). Acceptable web sites in the early season become unsuitable in the late season, perhaps because they have more extreme low tem-

peratures compared to other potential sites (Fig. 1). Web site selection in general may become more critical in the late fall when there is greater variation in temperature within a small geographic area (for example between shaded and sunny sites).

Other studies have shown that spiders have temperature-dependent web site preferences (e.g., Barghusen et al. 1997). In *N. clavipes*, Higgins and Ezcurra (1996) found evidence in high altitude tropical deserts that females build webs at higher distances from the ground during the late season, presumably as a means to control body temperature. However, here I demonstrate that temperature also varies within a single range of web heights (i.e., ≤ 2 m; Fig. 1), and females may respond to this variation through web site selection. Furthermore, the findings presented here show that females are at times exposed to temperatures prohibitively low for egg development. Future studies in *N. clavipes* should examine a broader range of temperatures, compare cyclical versus constant temperature regimes, and increase sample sizes in order to further describe how temperature affects egg development. The ability to overcome seasonal temperature constraints is particularly intriguing for a tropical species like *Nephila clavipes*, whose range has expanded to temperate areas. This species has undergone behavioral and life-history adaptations to the temperate climate; however, individuals may still suffer a late-season reproductive penalty due to insurmountable low temperatures.

ACKNOWLEDGMENTS

I thank Dan Hahn for help developing this project and for access to laboratory and incubator space. This work was funded by a National Science Foundation Doctoral Dissertation Improvement Grant (IOS-0909367). I thank the Ordway-Swisher Biological Station for use of their property in this study.

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Manuscript received 9 May 2011, revised 14 November 2011.